REMARKS

Applicants respectfully request reconsideration of the application in view of the foregoing amendments and the following remarks.

Claims 1-6, 9-29, 31 and 35-39 are currently pending. Claims 2, 4-6, 10-29, 31 and 35-39 have been canceled, without prejudice. Claims 1, 3 and 9 have been amended. Claims 40-56 have been added. Thus, after entry of the instant amendment, claims 1, 3, 9 and 40-56 will be pending. No new matter has been added.

Claim 1 has been amended to clarify the selection step in part (a) of the claim. As amended, the process for producing plasmid DNA in claim 1 comprises a first step of selecting a highly productive clonal subtype of a strain of *E. coli* transformed with a DNA plasmid. The highly productive clonal subtype is selected by first observing a phenotypic heterogeneity in a population of colonies generated by the transformed *E. coli* and then selecting as *potentially* highly productive clonal subtypes those colonies that represent a minor component of the recognized heterogeneity in said population of colonies. The potentially highly productive clonal subtypes are then purified. Finally, one of the purified, potentially highly productive clonal subtypes is selected as a highly productive clonal subtype after exhibiting a higher plasmid copy number per cell in comparison to non-selected, transformed *E. coli* subtypes of the same strain. Support for this amendment to step (a) can be found in claim 1 and on page 10, lines 12-16, of the specification. Claim 1 has also been amended to include a fermentation volume of greater than about 1000L. Support for this amendment can be found on page 18, lines 13-15, of the specification.

Claims 3 and 9 have been amended to correct a minor editorial error and change the dependency of the claims.

New claims 40-50 depend upon amended claim 1.

Claims 40 and 41 further define how phenotypic heterogeneity within the transformed *E. coli* is observed - by growing the transformed *E. coli* on blood agar (claim 40) at 30°C (claim 41). Support for these claims can be found in original claims 4 and 5.

Claim 42 further defines how the potentially highly productive clonal subtypes are selected – by selecting gray-colored colonies (which represent the minor component of the phenotypic heterogeneity) among a population of white-colored colonies (which represent the major component of the phenotypic heterogeneity). Support for this claim can be found on page 11, lines 23-28, of the specification.

Claims 43 and 47 further define how the selected, potentially highly productive clonal subtypes are purified. In claim 43, the selected, potentially highly productive clonal subtypes are purified directly from the blood agar. Support for this claim can be found in the sentence

spanning pages 12-13 of the specification. In claim 47, the selected, potentially highly productive clonal subtypes are purified from a second type of agar that does not contain blood products, wherein the colonies purified from said second agar correspond to the gray-colored colonies on the blood agar. Support for this claim can be found on page 13, lines 3-16, of the specification.

Claims 44-46 depend upon claim 43, and claims 48-50 depend upon claim 47. These dependent claims further clarify how the plasmid copy number per cell of the purified, potentially productive clonal subtypes is determined. Support can be found on page 17, lines 15-17, of the specification; page 20, lines 14-10, of the specification; and in original claims 11-16.

New claims 51-56 cover processes for the production of plasmid DNA that incorporate one or more of the steps outlined above. The support for these claims are as indicated above.

Rejection of Claims 10, 14-20 and 37-38 under 35 U.S.C. § 112, 2nd Paragraph

Claims 10, 14-20 and 37-38 were rejected for alleged indefiniteness under 35 U.S.C. § 112, 2nd paragraph.

Claim 10 was rejected for use of the phrase "industrial scale." Claim 10 has been canceled. However, the concept of industrial scale fermentation has been included in step (b) of amended claim 1, stating that the fermentation volume of step (b) is greater than about 1000L. Support for this amendment can be found on page 18, lines 13-15, of the specification. New claim 55 also incorporates this language.

Claims 14 and 18 were rejected for being unclear as to whether the claims cover a continuous or exponential fed batch fermentation. The subject matter of claims 14 and 18 has been deleted in the amended claim list.

In light of the amendments presented above, Applicants respectfully request the reconsideration of the instant rejection to claims 10, 14-20 and 37-38 under 35 U.S.C. § 112, 2nd paragraph.

Rejection of Claim 1 under 35 U.S.C. § 102

Claim 1 was rejected under 35 U.S.C. § 102(b) as being alleged anticipated by Chen et al. (IDS reference A). Chen et al. was cited as teaching a "fed-batch fermentation process for *E. coli* that produces a total yield of 10-fold the plasmid DNA as compared to manual fed-batch fermentation with the same plasmid transformed into the same *E. coli* strain" (p.3-4 of the Office Action). Chen et al. was previously cited against the original claims filed with this application to support the Office's conclusion that said claims did not possess a special technical feature.

Applicants argued that Chen et al. did not teach, as required by claim 1 of the original claims, a step of first selecting a highly productive clonal subtype of a strain of *E. coli* to cultivate in a fed-batch fermentation process. The outstanding Office Action states that this argument was not found persuasive because "prior selection is not required by the claims, therefore, Chen et al. do anticipate claim 1" (p. 2). Applicants respectfully traverse.

Chen et al. teaches an automated feed-back control, fed-batch fermentation process based on dissolved oxygen (DO) and pH for producing plasmid pENV in *E. coli* strain DH10B. This reference, however, does not teach the selection step included within claim 1.

As described in the application, Applicants have developed a process for producing plasmid DNA in *E. coli* with record high volumetric productivity in the order of 1 to 1.5 grams of plasmid/L. Applicants discovered that the transformation process can yield a heterogeneous population of *E. coli* clonal subtypes wherein the minor subset of this population demonstrates a higher specific productivity (copy number per cell) when compared to the major subset of the population. Thus, the claimed plasmid DNA production process includes a first step of selecting from this heterogeneous population a highly productive clonal subtype of transformed *E. coli*.

The selection step of claim 1 (step (a)) has been amended for clarification. First, "potentially highly productive clonal subtypes," a subset of the clonal subtypes that can be generated when transforming *E. coli* with a DNA plasmid, are identified when a phenotypic heterogeneity in the *E. coli* colony population is observed. The potentially highly productive clonal subtypes are the minor component of the heterogeneous population of colonies. Isolating clonal subtypes with the potential of being highly productive limits the amount of testing that is required to identify a highly productive strain. The purified, potentially highly productive clonal subtypes are then tested to determine which are highly productive (i.e., exhibiting a higher plasmid copy number per cell in comparison to non-selected, transformed *E. coli* clonal subtypes of the same strain). Once a highly productive clonal subtype is identified by the selection process described in step (a) of claim 1, it is cultivated using fed-batch fermentation in chemically-defined medium. Thus, claim 1 clearly comprises a selection step wherein a highly productive clonal subtype of transformed *E. coli* is selected from a heterogeneous pool of transformed *E. coli* clonal subtypes.

Chen et al. does not teach each of the steps of amended claim 1; thus, Applicants respectfully request the reconsideration of the instant rejection to claim 1 under 35 U.S.C. § 102(b).

Rejection of Claim 1-5, 11-13, 35 and 36 under 35 U.S.C. § 103

Claims 1, 2, 4, 11 and 12 were rejected under 35 U.S.C. § 103 for allegedly being unpatentable over Cress et al. (IDS ref.) in view of Korz et al. (IDS ref.). (Applicants note that it appears the Examiner intended to include claim 5 in this rejection.) Cress is cited for teaching a plasmid production method that includes "isolating chromosomal mutants of E. coli that maintain higher levels of an F' plasmid and cultivating them" (Office Action p. 4). The Office Action states that mutant strains were selected with "2-7 times more plasmids than unselected strains" and "cultured at 30°C for 24-30 hours" (p. 5). Korz et al. is cited for teaching a "fed-batch process for high cell density cultivation of E. coli" (Office Action p. 6). The website "microbelibrary.org" was also cited as demonstrating that "E. coli cells grown on blood agar are phenotypically gray" (Office Action p. 6). Thus, the Office Action concludes that one of ordinary skill in the art would have been motivated to combine Cress et al., teaching higher levels of F' plasmid, and Korz, teaching fed-batch fermentation, to "maximize the volumetric productivities of bacterial cultures" (p. 6). Applicants respectfully traverse.

Step (a) in claim 1 has been amended to clarify the selection step of the claimed plasmid DNA production process, described in detail above. This selection step is not described or suggested by Cress et al.

Claim 1 has also been amended in step (b) to include that the fermentation volume for cultivating the highly productive clonal subtype is greater than about 1000L. As stated above, the plasmid DNA production process described in the instant application generates record high volumetric productivity and is extremely useful when producing plasmid DNA at an industrial scale (defined therein as fermentation volumes of greater than about 1000L) for applications where large volumes of plasmid DNA are desired (e.g., manufacturing polynucleotide vaccines). Cress et al. states that the copy number of the F plasmid is usually 1-2 plasmids per replicating chromosome, and the increased copy number of the F'*lacI, P pro*$^{+}$ plasmid identified therein is at most four times the copy of the parental strain. Thus, one of skill in the art would not have considered the F plasmid system or a selection process specific for that system, as described in Cress et al., for generating extremely large volumes of plasmid DNA. Indeed, large scale preparations of the F plasmid are not described or contemplated in this reference. Thus, one of skill in the art would not look to Cress et al. as a viable 35 U.S.C. § 103 teaching related to the claimed invention.

Korz et al. does not cure the deficiencies outlined above in Cress et al. Korz et al. describes a fed-batch fermentation technique for *E. coli* tested in a 5L bioreactor. The bacteria cultivated in Korz et al. do not contain a DNA plasmid, and there is no discussion of the scalability of this technique or the applicability to plasmid DNA production.

Claim 3 was rejected under 35 U.S.C. § 103 as being allegedly unpatentable over Cress et al. and Korz et al., as described in the above rejection, in further view of Mason et al. (IDS ref.). Mason et al. was cited for teaching "DH5α cells and increasing copy number of plasmids" (Office Action p. 7). Applicants respectfully traverse.

Applicants argue that Mason et al. does not cure the deficiencies outlined above of Cress et al. and/or Korz et al. Nothing in Mason et al. provides information on the production of a DNA plasmid on an industrial scale or the selection step of claim 1.

Claims 13, 35 and 36 were rejected under 35 U.S.C. § 103 as being allegedly unpatentable over Cress et al and Korz et al., as described in the above rejection, in further view of Zhang et al. (IDS ref.). Applicants respectfully traverse. However, in efforts to speed prosecution, claims 13, 35 and 36 have been canceled, without prejudice.

In light of the arguments and amendments presented above, Applicants respectfully request the reconsideration of the instant rejections under 35 U.S.C. § 103.

In view of the amendments and comments herein, Applicants respectfully take the position that all claims are in proper form for allowance and earnestly solicit a favorable action on the merits. The Examiner is invited to contact the undersigned attorney if clarification is required on any aspect of this response, or if any of the claims are considered to require further amendment to be placed in condition for allowance after entry of this Amendment.

Respectfully submitted,

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